Medical Record

AN INTERNATIONAL JOURNAL OF MEDICINE AND SURGERY

Founded 1843

Vol. 163, No. 2 FEBRUARY, 1950 Whole No. 2804

CONTENTS

PAGE

PROBABLE ROLE OF SUCCINIC ACID IN BACTERIOSTASIS BY SUCCINYLSULFATHIAZOLE
Michele Gerundo, M.D., Ph.D. 23

INFLUENCE OF TEMPERATURE AND MEDIUM UPON THE ACTION OF SULFONAMIDE
Michele Gerundo, M.D., Ph.D. 35

SUSTAINED BLOOD STREPTOMYCIN LEVELS FOLLOWING DEPOT ADMINISTRATION OF THE FERRIHEME AND OLEOCARBONATE SALTS
Robert D. Barnard, M.D. 40

NEWS, NOTES AND EDITORIAL COMMENTS 43

PUBLISHED MONTHLY BY

WASHINGTON INSTITUTE OF MEDICINE

1708 Massachusetts Ave., Washington 6, D. C.

MEDICAL RECORD

AN INTERNATIONAL JOURNAL OF MEDICINE AND SURGERY

Founded 1845

PUBLISHED BY

WASHINGTON INSTITUTE OF MEDICINE

1708 Massachusetts Ave., N. W. Washington 6, D. C.

Executive Editor
HENRY J. KLAUNBERG, Ph.D.

EDITORIAL BOARD

Dermatology
DR. HERMAN GOODMAN
New York, N. Y.

Diagnosis
DR. JOSEPH H. ISENSTEAD
Canajoharie, N. Y.

Endocrinology
DR. I. SIMON
Paris, France

Gastroenterology
DR. W. B. JAFFE
Detroit, Mich.

Gynecology
DR. CHARLES MAZER
Philadelphia, Pa.

History of Medicine
DR. SOLOMON R. KAGAN
Roxbury, Mass.

DR. MAX NEUBURGER
Buffalo, N. Y.

Mental Hygiene
DR. B. LIBER
New York, N. Y.

Metabolism
DR. ANTHONY SINDONI, JR.
Philadelphia, Pa.

Microbiology, Pathology
DR. M. GERUNDO
Lubbock, Texas

Neuropsychiatry
DR. EDWARD PODOLSKY
Brooklyn, N. Y.

Otolaryngology
DR. ABRAM H. PERSKY
Philadelphia, Pa.

Otorhinolaryngology
DR. IRVING W. VOORHEES
New York, N. Y.

Pediatrics
DR. I. NEWTON KUGELMASS
New York, N. Y.

Psychosomatic Medicine
DR. A. D. JONAS
New York, N. Y.

Radiology
DR. IRA KAPLAN
New York, N. Y.

Urology
DR. JAMES F. MCCAHEY
Philadelphia, Pa.

Surgery
DR. JOHN T. MCLAUGHLIN
Altadena, Cal.

Entered as Second Class Matter at the Post Offices at Philadelphia, Pa. and Washington, D. C. under the Act of March 3, 1879. Editorial and Executive Offices, 1708 Massachusetts Ave., Washington 6, D. C.

Copyright, 1949, by Washington Institute of Medicine.

Subscription rate $5.00 per year, 3 years $10.00
(No additional postage for foreign subscriptions)

MANUSCRIPTS

Illustrations: Photographs should be numbered and bear on the back author's name and title of article; top of the picture should be indicated. Glossy prints produce the best half-tones. Drawings and charts for reproduction should be made in black ink.

Bibliographies should follow the style of the Quarterly Cumulative Index Medicus of the American Medical Association, and should give name of author, title of article, name of periodical, with volume, page, month, day of month if weekly, and year.

Manuscripts should carry addresses of authors and should not be rolled.

Reprints are available. Prices can be secured after article is in type.

All material published in the MEDICAL RECORD is copyrighted and should not be reproduced without permission.

All material for publication should be sent to THE EXECUTIVE EDITOR of the MEDICAL RECORD, 1708 Massachusetts Ave., Washington 6, D. C.

Exclusive publication: Original articles are accepted with the understanding that they are contributed only to the MEDICAL RECORD.

Preparation: Material must be typewritten and double spaced. Carbon copies or single spaced manuscripts will not be considered

How will they look to YOU a few years from now?

Your wife's eyes: What will you read in hers when she asks whether you can afford that modest cottage that's for sale?

Your boy's eyes: What will you see in his eyes the day he asks whether you can afford to send him to college?

Your own eyes: What will the mirror tell you about them when it's time to retire, and take things easier?

There's no better time than right now to sit back and think what *you* will see in your family's eyes a few years from now.

Whether they glow with happiness or turn aside with disappointment depends, to a very large extent, upon what you do *now*.

So plan *now* for that home you plan to buy eventually . . . set aside money *now* for his college education . . . plan *now* for the day you can retire.

Decide now to put part of your salary week after week, year after year in U. S. Savings Bonds, so that you will have the money for the *important* things you and your family want.

Insure your future by signing up on the Payroll Savings Plan where you work, or the Bond-A-Month Plan where you have a checking account.

Chances are you won't miss the money now, but you certainly will a few short years from now if you haven't got it!!

P. S. Remember, too, that every $3 you invest now in U. S. Savings Bonds returns $4 to you in just ten short years.

Automatic saving is sure saving — U.S. SAVINGS BONDS

Contributed by this magazine in co-operation with the Magazine Publishers of America as a public service.

Probable Role of Succinic Acid in Bacteriostasis By Succinylsulfathiazole

MICHELE GERUNDO, M.D., PH.D.

From the Department of Bacteriology, University of California, Los Angeles
and
The Department of Laboratories, St. Francis Hospital, Honolulu, T. H.

EXPERIMENTAL

The action of Succinylsulfathiazole or "Sulfasuxidine" *in vivo* is so different from its effect *in vitro*, as shown by clinical reports, that it was felt necessary to examine separately the function of succinic acid which is coupled with the sulfathiazole molecule. Succinic acid may be considered

TABLE 1

SYNTHETIC MEDIUM OF SARYUN AND BEARD (A11), CONTAINING IN ADDITION SODIUM SUCCINATE AND GLUCOSE-SULFATHIAZOLE 1:1000

	With Sulfathiazole		*Without Sulfathiazole*	
	Sodium Succinate	*Sodium Succinate and Glucose*	*Sodium Succinate*	*Sodium Succinate and Glucose*
Proteus ox19	?	?	*x*	xx
Proteus x19	?	?	x	
Para B.	*x*	x	xx	x
Salm. ab. ovis	–		no growth	
aerog. Aerob.	x	*x*	xx	x x
Para. A.			no growth	
Shig dys.	–		no growth	–
Eberth. typhi	–		no growth	
Flexner	–	–	*x*	xx
	–	–	xx	xx
Strepto. fecalis	–	–	no growth	
Shig. alkal.	–	–	?	xx
	–	–	xx	
Coli communior 15	–	–	xx	xx
Pseudomonas 2	*x*	x	x	xx
	xx	xx	xxx	xxx
Alkalig. 1	–	–	xx	*x*
	–	–	xxx	–
Alkalig. 110	–	–	x	–
	–	–	x*x*	x
Proteus vulg.	–	–	x	xx
	–	–	xxx	xxx
Bruc. Ab.	–		no growth	–
Staphylococcus 12			no growth	–
Sonne	xx	xx	xx	xx
	–	–	xxx	xxx
Bruc. Suis	–		no growth	–
Proteus Su 30 (1)	*x*	*x*	*x*	x*x*
Alkalig. 110 Su4 (1)	?	?	?	x
Proteus Su5 (1)	?	*x*	?	x
Pseudom. Su31 (1)	x	x	xx	xx
	xx	xx	–	xxx
Shig. Dys. Su2 (1)	–		no growth	–
Aerog. Aerob. (1) Su31	xxx	xxx	xx*x*	xxx

(1) These strains have been cultured in Sulfasuxidine containing medium for the number of passages indicated after Su.

as derived from two molecules of acetic acid. According to studies with heavy carbon, the typically reversible reaction catalyzed by succinic dehydrogenase is indicated by the following:

$$COOH-CH_2-CH_2-COOH \longrightarrow COOH-CH-CH-COOH+2H$$

In the first experiment, the nutritive value of the heart infusion medium was reduced to one-half the usual amount in order to facilitate recognition of the bacteriostatic activity of the drug which was present in a concentration of 1:1000. With a concentration of 0.5 per cent succinic acid in the medium, the growth was practically nil, even in absence of "Sulfasuxidine," when compared to that obtained in presence of 0.5 per cent glucose.

The results were so striking that, in order to exclude any technical error, fresh medium was prepared on two occasions, controlled carefully as to *p*H, time of sterilization and age of culture, but the results obtained were the same. At least in such concentration, inactive succinic acid is capable of inhibiting growth *per se*. Subcultures from succinic acid tubes were uniformly sterile.

Considering that the inhibitory action of "Sulfasuxidine" might be due to the presence of free succinic acid, sodium succinate in the concentration of 1 per cent was used in the next experiment in which glucose was

TABLE II

H1 BH1

HEART INFUSION BROTH *p*H 7.3
SULFATHIAZOLE 1:2000
SUCCINIC ACID 0.2 PER CENT
PABA 1:1000 5CC. FOR EACH 100CC. OF MEDIUM

	With Sulfathiazole		*Without Sulfathiazole*	
	Succinic Acid	*Succinic PABA*	*Succinic Acid*	*Succinic PABA*
Bruc. Suis	–	x	x	x
Fiexner	–	x	xx	xx
aerog. Aerob.	–	xx	xx	xxx
Bruce. Ab.	–	x	x	x
Shig. Alk.	–			x
Alkalig. 110	–		x	xx
Staphylococcus 12	–		x	x
Proteus vulg.	–	xxx	xxx	xxx
Strepto. fec.	–	x	xx	xx
Pseudom.	xx	xx	xx	xx
Ae 100 (salm.)	x	xx	xx	xx
Alkaligenes	x	x	xx	xx
Prot. Su32	–	xx	xx	xx
Alkal. 110 Su6	–	–	–	–
Prot. Su32	–	xx	xx	xx
Prot. Su6	–	xx	xx	xx
Prot. Ox19	–	no growth		
Prot. x19	–	no growth		
Salm. ab. ovis	–	no growth		
Para A	–	no growth		
Sonne	–	no growth		
Para B	–	no growth		
Shig. dys.	–	no growth		
Eberth. typhi.	–	no growth		

added to one-half of the medium (Synthetic Medium A-11). From Table I it is evident that sodium succinate supported the growth of a number of bacteria, but the growth was not so abundant as when glucose was also present. In the tubes containing sulfathiazole, bacteriostasis was evident, but, at least in this experiment, glucose added to sodium succinate failed to exercise any protective influence upon the growth. It is noteworthy to mention at this point that many bacteria did not grow at all on this synthetic medium, particularly those which, from previous experiments, had proved to be very susceptible to the action of "Sulfasuxidine." For this reason the use of a synthetic medium was reserved only for special experimental conditions and a heart infusion broth diluted to one-half its usual strength, was then adopted throughout the series of experiments. When methylene blue was added to the medium as an indicator of possible transfer of hydrogen, it was noted that decolorization of the dye was nil in the tubes containing sulfathiazole; whereas, it was complete or at least partial in the tubes containing sodium succinate or glucose without sulfonamide.

Para-amino-benzoic acid (Paba) was used in conjunction with succinic acid 0.2 per cent concentration, which permits a certain amount of growth, and sulfathiazole (Table II). With many bacteria, as seen from the table, Paba antagonized the bacteriostatic action of sulfathiazole but with others,

TABLE III

HB5

Heart Infusion Broth *p*H 7.3
Succinic Acid 0.2 per cent
Thiamin 1:10000
Sulfathiazole 1:4000
Glucose 0.5 per cent

	With Sulfathiazole		*Without Sulfathiazole*	
	Succinic Acid Thiamin	*Succinic Acid Thiamin Gluc.*	*Succinic Acid Thiamin*	*Succinic Acid Thiamin Gluc.*
Para B.	x	x	xx	xxx
Eberth. typhi	f	xxf	xx	xxx
Flexner	\	xf	xf	xxx
Coli comm. 10	\	xf	xx	xxx
Pseudomonas	f	x	xx	xxx
Shig. Alkal.	f	xxx	xx	xxx
Shig. dys.	\	xx	xf	xxx
Strepto. fec.	–	xxx	x	xxx
Bruc. Suis	–	–	f	f
Prot. x19	f	xxx	xx	xxx
Ae 100 (Salm.)	f	xxx	f	xxx
Pseudom.	\	x	xx	xf
Sonne loc.	f	xx	xf	xxx
Alkalig. 110		–	f	f
Staphylococcus 12		–	x	xf
aerog. Aerob.	f	xxf	xxf	xxx
Para A.	f	xx	f	xxx
Alkalig. 1	\	x	xxx	xxx
Coli communior 15	\	x	xxx	xxx
Proteus Su31	\	xxx	x	xxx
Alkal. 110 Su5	–	–	–	–

like *Shigella alkalescens*, *Alkaligenes*, *Staphylococcus*, and to a certain degree *Streptococcus fecalis*, it failed to promote growth, most probably because growth inhibition was due to the presence of free succinic acid.

The addition of thiamin chloride to sodium succinate in the medium did not alter the results. The differences observed were so small that, form the experimental point of view, they were insignificant. However, when glucose was added to the medium as is shown in Table III, thiamin chloride stimulated a good growth in presence of glucose, even in the tubes not containing sulfathiazole. When using a concentration of 0.2% succinic acid and 1:4000 sulfathiazole, 0.5% glucose added to the medium antagonized to a certain extent the succinic acid-sulfathiazole inhibition (Table IV). This role of glucose was in accord with previous findings.

The next table (Table V) shows the results obtained with the same medium as in Table IV, with the exception that in this experiment disodium phosphate was added. The two experiments were run simultaneously and the tubes were inoculated from the same cultures and with the same loop. As all the cultures were in fluid media, and the tubes were shaken before inoculation, it is fair to assume that the inocula contained a somewhat similar number of bacteria for each given culture. By comparing the two tables, it is clear that the presence of phosphate enhanced to a small extent sulfonamide inhibition in absence of glucose. It has been pointed out by some authors that sulfur is not entirely essential to the action of sulfon-

HB6

TABLE IV

HEART INFUSION BROTH
SUCCINIC ACID 0.2 PER CENT
SULFATHIAZOLE 1:4000
GLUCOSE 0.5 PER CENT

	With Sulfathiazole		*Without Sulfathiazole*	
	Succinic Acid	*Succinic Acid Glucose*	*Succinic Acid*	*Succinic Acid Glucose*
Proteus vulg.	x	x	xx	xxxx
Staphylococcus	–	–	x	xx
Pseudomonas	f	f	xf	xf
Flexner	f	xx	x	xxx
Coli communis 10	x	xx	xf	xxxx
Strepto fec.	f	xx	f	xxx
Shig. dys.	f	f	f	xxx
Shig. alkal.	x	x	x	xx
Alkalig. 1	x	f	xxx	xxx
Ae. 100 (Sal.)	x	xx	x	xxx
aer. Aerob.	f	xx	xf	xxx
Prot. x19	xf	xxx	xxf	xxx
Eberth. typhi	x	xxf	xxx	xxx
Sonne	x	xx	x	xxx
Para B.	x	xx	xxx	xxx
Alkalig. 110	–	–	x	xf
Coli Communior 15	x	xx	xf	xxx
Bruc. Suis	–	–	f	f
Para A.	x	xx	x	xxx
Porteus Su31	xxf	xxf	xxf	xxf
Alkal. 110 Su5	–	–	x	xf

amides and that any other metal could serve the same purpose as sulfur. It is possible that in this case the presence of phosphate without glucose acted to enhance the sulfonamide action. The two strains of *Esch. coli* used in the experiments showed, however, less growth in presence of phosphate than they did in its absence when glucose was added to the formula. Whether this finding is due to the fact that *Esch. Coli* is capable of metabolizing glucose without previous phosphorylation is speculative and to it further study should be devoted.

Sulfadiazine in a concentration of 1:4000 was next used in conjunction with succinic acid. As is evident from Table VI, the succinic acid-sulfadiazine combination developed more perfect bacteriostasis than did sulfadiazine alone. Glucose exerted the same protective role upon bacterial growth as already has been noted in a previous section. That this role was not antagonistic, is clear from the fact that the influence was not noticed on the growth of bacteria not fermenting nor utilizing glucose for their metabolism. The same applies to strains of *Pseudomonas*, *Alkaligenes*, and *Brucella*. On the other hand, it was not due to non-specific growth-stimulating influence, since control tubes without glucose showed practically the same amount of growth as did the control tubes containing glucose.

Almost identical findings were obtained when sulfaguanidine was used

TABLE V

HB7

(Same as HB6, except that the medium contains Phosphate)

HEART INFUSION BROTH *p*H 7.3
SUCCINIC ACID 0.2 PER CENT (in all the tubes)
DISODIUM PHOSPHATE 0.25 PER CENT (in all the tubes)
SULFATHIAZOLE 1:14000
GLUCOSE 0.5 PER CENT

	Succinic Acid	*Succinic Acid Glucose*	*Succinic Acid*	*Succinic Acid Glucose*
Proteus vulgaris	*r*	*r*	XX	XXX
Staphylococcus 12	–	–	X	XX
Pseudomonas	*r*	*r*	X*r*	X*r*
Flexner	X	XX	X	XXX
Coli communis 10	X	X	XX	XXXX
Strepto fec	*r*	XX	*r*	XXX
Shig. dysent	–	–	X	XXXX
Shig. alkal	*r*	*r*	X	XXX
Alkalig. 1	*r*	?	XXX	XXX
Ae. 100	X	XX	X	XXX
aer Aerob	*r*	XX	X*r*	XXXX
Prot. x19	X*r*	X*r*	XXX	XXXX
Eberth. typhi	X	XX*r*	XXX	XXX
Sonne	*r*	XX	X	XXX
Para B	X	XX	XX*r*	XXX
Alkalig. 110	–	–	X	XX
Coli communior 15	X	X	X*r*	XXXX
Bruc. Ab	–	–	*r*	*r* (1)
Bruc. Suis	–	–	*r*	*r*
Para A	X	XX	X	XXXX
Proteus Su31	X*r*	XX*r*	XX*r*	XX*r*
Alkal. 110 Su5	–	–	X*r*	X*r*

(1) It did not grow in tubes HB6 containing no Phosphate.

in similar tests. Succinic acid enhanced the bacteriostasis of sulfaguanidine, though not to the same extent as it did sulfadiazine and sulfathiazole: glucose exerted the same protective action upon bacterial growth. In this experiment two concentrations of glucose were used, 0.5% and 0.1%. There was no difference in amount of growth between the two sets of tubes, indicating that even a concentration of 0.1% glucose, as usually found in body tissues, may protect a glucose fermenting bacterium from the unfavorable action of sulfonamide compounds.

When one considers that succinic acid is oxidized to fumaric acid by many bacteria, the role of this acid upon bacterial growth then came for consideration. The results showed that fumaric acid had an action upon bacterial growth similar to that of succinic acid. The addition of glucose to fumaric acid favored the growth of most of the bacterial strains used in the experiment, though when glucose was used alone in concentration of 0.5% and 0.1%, growth was better than when glucose and fumaric acid were added together. The exception here was a strain of Staphylococcus which was inhibited by a concentration of 0.1% fumaric acid alone. Susceptible strains in general showed no reduction of methylene blue in presence of fumaric acid and sulfathiazole.

TABLE VI

HB8

SD – Sulfadiazine in Heart Infusion Broth without addition.

HEART INFUSION BROTH *p*H 7.8
Sulfadiazine 1:4000
SUCCINIC ACID 0.2 PER CENT
GLUCOSE 0.5 PER CENT

	With Sulfadiazine			*Without Sulfadiazine*		
	SD	*Succ. Ac.*	*Gluc. Suc. Ac.*	*Gluc.*	*Gluc.*	*No Gluc.*
Prot. vulg.	–	–	xf	xx	xxx	xxx
Staphylo. 1¢	x	–	–	x	xf	xxx
Pseudomonas	x	–	f	x	xxx	xxx
Flexner	x	f	xxx	xxf	xxx	xxx
Coli. comm. 10	xf	f	xxx	xxx	xxx	xxx
Strepto. fec.	x	–	xxxx	xxx	xxf	xx
Shig. dysent.	–	–	–	x	xx	xf
Shig. alkal.	x	–	x	xx	xxxx	xxf
Alkalig. 1	x	x	x	xf	xxxx	xxxx
Ae. 100	x	–	xx	xxx	xxx	xxx
aer. Aerob.	xx	x	xxx	xxx	xxxx	xxx
Prot. x19	x	–	xxx	xxf	xxx	xxx
Eberth. typhi	x	–	xxx	xxf	xx	xx
Sonne	x	–	–	xxx	xxf	xx
Para B.	x	x	xf	xx	xxf	xxx
Alkalig. 110	–	–	–	–	xx	xf
Coli communior 15	x	–	xf	xx	xxx	xxf
Bruc. Ab.	–	–	–	–	–	–
Bruc. Suis	–	–	–	–	x	x
Para A.	x	f	xxx	xxx	–	–
Prot. Su31	xxx	xx	xxf	xxxx	xxx	xxx
Alkal. 110 Su5	–	–	–	–	xx	xf
Proteus Su6	xx	xxx	xxx	xxxx	xxx	xxf

When sodium lactate was used as a substrate (Table VII), typical sulfonamide inhibition was observed. Thiamin added to the sodium lactate did not apparently exert any protective role upon growth either in presence of sulfathiazole or in its absence. As all the strains in the experiment utilized sodium lactate, with the exception of a strain of *Proteus Vulgaris* which had been grown through 31 passages in presence of sulfasuxidine, it seemed logical to conclude that sulfonamide prevented the bacteria from utilizing lactate as a source of energy.

Discussion

The previous experiments prove beyond doubt that the addition of succinic acid or its immediate products will enhance the action of sulfonamide upon bacteria. Some strains which can dehydrogenate succinic acid may defintiely escape its inhibitory action and thus may survive.

Sulfasuxidine which is succinyl sulfathiazole, comes under the influence of varied bacterial flora in the large intestine, where it is split into sul-

TABLE VII

HB3

Heart Infusion Broth
Sodium Lactate 0.4 per cent
Thiamin 1:10000
Sulfathiazole 1:4000
Methylene Blue 1:5000 - 2cc. for each 100cc. of medium

	With Sulfathiazole		*Without Sulfathiazole*	
	Sodium Lact.	*Sodium Lact. and Thiamin*	*Sodium Lact.*	*Sodium Lact and Thiamin*
Coli Communis 10	x	x	xx	xx
	–	–	c. d.	c. d.
Prot. x19	x	x	xx	xx
	p. d.	c. d.	c. d.	c. d.
Para B	x	x	xx	xx
	–	–	p. d.	p. d.
Alkalig. 110	–	–	x (1)	x (1)
Coli communior 15	–	–	x	x
	–	–	p. d.	p. d.
Eberth. typhi	x	x	x	x
Streptococcus fec.	–	–	–	–
Para A	–	–	x	x
Bruc. Ab.	–	–	–	–
Flexner	x	x	xx	xx
Shig. Alkal.	x	x	xx	xx
	–	p. d.	p. d.	c. d.
Staphylococcus 1#	–	–	–	–
Shigella dys	–	–	–	–
Bruc. Suis	–	–	–	–
Prot. Ox19	–	–	–	–
aerog. Aerob.	x (1)	x (1)	xx (1)	xx (1)
	–	–	p. d.	p. d.
Salmonella sp.	–	–	xx	xx
	–	–	p. d.	p. d.
Alkaligenes 1	x	x	xx	xx
	–	–	p. d.	p. d.
Prot. vulg.	x	x	xx	xx
	–	–	p. d.	p. d.

(1) Delayed growth

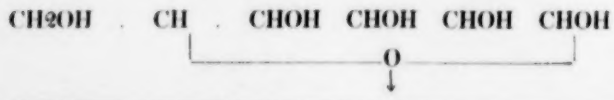
fathiazole and succinic acid. The action of sulfasuxidine appears to be due to the combined action of succinic acid and sulfathiazole. Whether free succinic acid has any influence upon the *p*H of the contents of the colon has not been investigated. When the acid is combined with any one of the sulfonamides, the results demonstrate increased inhibitory activity of bacterial growth. The conjugated compound is not effective as when the components, succinic acid and sulfathiazole, are both free. Some of the bacteria capable of dehydrogenating succinic acid may in their turn be susceptible to sulfathiazole. We may divide bacteria according to their susceptibility, as follows: (1) bacteria which are sensitive to the conjugated drug; (2) bacteria which are inhibited by succinic acid and by sulfathiazole; (3) bacteria which are inhibited by sulfathiazole, not by succinic acid; (4) bacteria which are not inhibited by either succinic acid or by sulfathiazole. These last are capable of dehydrogenating succinic acid and possibly of oxidizing sulfathiazole to an inactive compound.

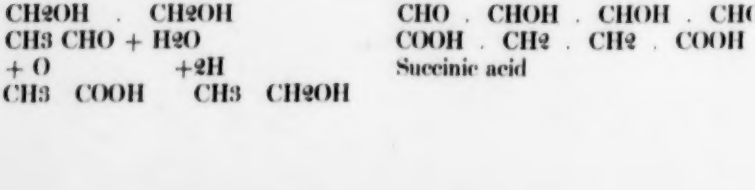
Succinic acid enters into the biologic oxidation cycle of Szent-Gyorgy[2]: Succinic acid ⟷ Fumaric acid ⟷ Malic acid ⟷ Oxaloacetic acid. It may arise from glucose if 0.5 per cent $NaHCO_3$ saturated with 5 per cent CO_2 is used[3], but it is not formed in presence of 1 per cent KH_2PO_4. Although it may originate also from aminoacids of the dicarboxylic series (glutaric and aspartic), Quastel and Woolf[4] admit a reversible reaction: l-aspartic acid ⟷ fumaric acid plus ammonia, and a reduction of fumaric acid to succinic acid by action of bacteria.

Originally an oxidation of acetic acid was postulated to account for succinic acid:[5]

$$\begin{matrix} CH_2-COOH & & CH_2-COOH \\ + & \longrightarrow 2H + & \\ CH_2-COOH & & CH_2-COOH \end{matrix}$$

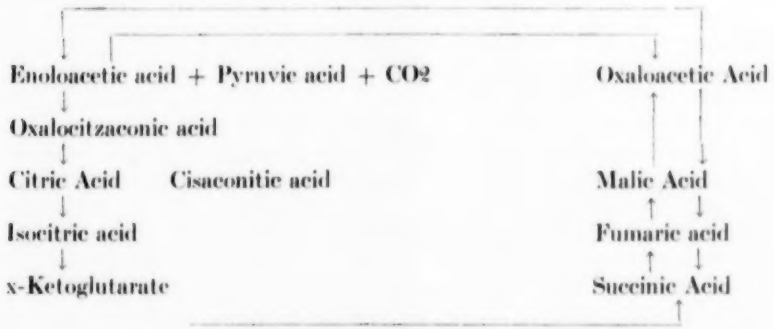
It has been suggested however, by Virtanen and Scheffer[6] that a cleavage of the hexose molecules into C_2 and C_4 compounds under the influence of organisms may lead to the formation of acetic and succinic acid and ethyl alcohol. The scheme of the two authors is as follows:





There is now evidence available which indicates that CO_2 plays a very important role in the formation of succinic acid. Elsden[7] demonstrated that when a washed suspension of *Esch. Coli* ferments pyruvate, the rate of formation of succinic acid is raised by 50% on increasing the CO_2 content of the medium.

The citric acid cycle, into which succinic acid fits, can be represented according to Wood and Werkman's modification of Szent-Gyorgyi's scheme in this way:[8]



Barron[9] in a study of the comparative rate of utilization of pyruvate, citrate and α-Ketoglutarate showed that *Pseudomonas aeruginosa* and *Neisseria gonorrheae* are unable to utilize citrate or α-Ketoglutarate but that they are able to utilize pyruvate formed through a phosphorylating cycle.[10]

When it became evident that the approach to the mechanism of action of sulfasuxidine was to study separately and jointly the action of the two components of the drug, namely, succinic acid and sulfathiazole, a synthetic medium, which contained some definite constituents, was considered to be advisable. Muir, Shamleffer and Jones[11] recommended a formula similar to Simmon's citrate medium for the study of the anti-bacterial effect of sulfathiazole. However, the synthetic medium used did not support good growth and in such a medium as already pointed out elsewhere, sulfonamide action was more strong.

A recent contribution to the subject of sulfasuxidine which appeared when our experimental work had already been completed and the conclusions reached, is that by Poth and Ross[12] who investigated the relationship between the rate of hydrolysis and bacteriostasis by succinyl sulfathiazole. They found that the velocity of hydrolysis in an acid solution frequently parallels the bacteriostatic activity but that the rate of hydrolysis in basic solution gives no indication of the bactericidal action. They concluded that the entire antibacterial activity of conjugated sulfonamides, such as succinyl sulfathiazole, is not due solely to the action of free sulfonamides

produced by hydrolysis. Furthermore, they pointed out that the activity may be influenced by formation of split products not containing the sulfonamide radical, such as succinate from succinyl derivatives. According to them, the biologic activity of the drug is not entirely explained and the complex mode of action cannot be evaluated at the present time. Our results confirm and supplement their findings. Cultural experiments in our hands proved in fact that succinic acid added to a sulfonamide compound enhances its action to a great extent although it is not in itself growth-inhibitory for that particular strain.

Den Dooren de Long[13] carried out a large number of experiments on nutritional requirements of the bacteria and found that *B. aerogenes* utilizes succinamide, but that *Esch. coli* cannot do so. Braun, Cahn and Bronner[14] demonstrated that *Eberth. typhi* on synthetic medium is unable to utilize succinic acid as a source of carbon, and this we confirm.

Qaustel[15] cultivated a number of bacterial organisms on ammonium fumarate and ammonium succinate which were found to be an excellent source of nutrition to urany forms. The table is reproduced here completely since it serves to support some of our findings:

	Ammonium Succinate	*Ammonium Fumarate*
Sarcina aurea	No growth after 24 days	No growth
B. pyocyaneus	Extensive surface growth	Same, except lighter pigment
B. subtilis	No growth	
B. fluorescens	Excellent	Excellent
Proteus vulgaris	Slow but perceptible	Good growth
Staphylococcus sp.	Poor	Poor
B. coli communis	Very good	Very good

In our experiments we have found that *Proteus vulgaris*, *Pseudomonas* and some strains of *Esch. coli* grew well or moderately well in the presence of succinic acid, but that *Staphylococcus* was inhibited.

McLeod and Gordon[16] some years ago made an attempt to classify bacteria according to their ability to reduce glutathione. By using a nitroprusside test, they found that Para B and Cholera Vibrio gave the strongest reactions but *Staphylococcus* and *Streptococcus* produced no change. On the basis of the experimental findings, it was concluded that oxidatized glutathione originally present in the medium is reduced by the bacteria in the course of their growth. Glutathione under anaerobic conditions, accelerates oxidation necessary to normal metabolism. The authors distinguished three groups of bacteria according to biochemical behavior: 1) strong reducers, *Para B.*, *Cholera Vibrio*, *B. pyocyaneus*, *B. Gartner*, and anaerobes; 2) moderate reducers, some strains of *Esch. coli*, *Eberthella typhosa*, *Proteus;* 3) non-reducers: *Staphylococcus aureus*, and

albus, Neisseria gonorrheae, Neisseria meningitidis, Diplococcus pneumoniae. Hemophilus influenza, B. Morax-Axenfeld, B. paratyphosus A, all of the dysentery bacilli, *Streptococcus pyogenes.*

More recent work[17] has again stressed the importance of reduced glutathione for the activity of succinic dehydrogenase. It seems natural to link the two functions together and to advance the hypothesis that some bacteria are unable to dehydrogenate succinic acid because they lack the reducing capacity upon glutathione, moreover, the presence of oxidized glutathione definitely inhibits the action of the dehydrogenases. Comparng the findings of McLeod and Gordon on reduction of glutathione on one hand, and on the other, the susceptibility to sulfonamides of the strains used in our experiments, one notes that bacteria belonging to non-reducers group were definitely more susceptible to the drug whereas strong reducers, like the *Salmonellae*, were more resistant to it. The exception of *Salmonella typhi*, which in our experiments proved to be somewhat sensitive to sulfonamide, finds a parallel in McLeod and Gordon's finding that it is a non-reducer. The fact that *Esch. coli, Proteus vulgaris* and *Eberth. typhosa* were classified as moderate reducers by McLeod and Gordon correlates with our finding, namely, that *Esch. coli* and, to a certain extent, *Proteus vulgaris*, were somewhat susceptible to this action of sulfonamide.

We are planning to repeat these experiments and to correlate them with inhibition by succinic acid and sulfonamide, as doubtless there is a connection between the mechanism of sulfonamide and the redox system of bacteria.

It is interesting to observe that Trefouel, Nitti and Bovet[15] while attempting to correlate chemical structure and chemotherapeutic activity of the organic derivatives of sulphur, found that all the sulphur derivatives (sulphides, sulfoxydes) with bacteriostatic properties, contain highly oxidized sulphur. These derivatives are characterized also by the radical which is common to both sulfamides and sulfones.

It is true that new investigations[19] have afforded evidence that the sulfur of active compounds can be replaced by other elements such as arsenic, phosphorus or tellurium. Collier[20] believes that the polarity of the molecule is increased by the substitution of sulfur with other elements probably through intensification of the active para-amino group.

From our findings as well as from those of other investigators, it does

seem clear that organic acids are capable of intensifying the bacteriostasis by sulfonamides, if they themselves are not already inhibiting growth.

BIBLIOGRAPHY

1. Hutton, D., Slade, C. H., Werkman, C. H., Bacterial assimilation of acetic and succinic acids containing heavy carbon by Aerob. indologenes, *Proc. Soc. Exper. Biol. & Med. 51*:65, 1942.
2. Oppenheimer, C. and Stern, K. G., *Biological Oxidations*, Junk, Publisher, The Hague, 1939.
3. Kleinzeller, A., Formation of succinic acid in yeast, *Bioch. J., 35*:494, 1941.
4. Quastel, J. H. and Woolf, B., Equilibrium between l – aspartic acid, fumaric acid and ammonia, *Bioch. J., 20*:545, 1926.
5. Stephenson, M., *Bact. Metabolism*, p. 93, Longmans, Greene Co., London, 1943.
6. Quoted in Stephenson, *ibid.*
7. Elsden, S. R., Effect of CO_2 on the production of succinic acid by B. Coli, *Bioch. J., 32*:187, 1938.
8. Barron, E. S. G., *Mechanism of carbohydrate metabolism in Adv. in Enzym.*, Vol. III, 149–184, 1943.
9. Barron, E. S. G., Loc. cit.
10. James, W. O., James, G. M., Bunting, A. H., On the method of formation of pyruvic acid by barley, *Bioch. J., 35*:588, 1941.
11. Muir, R. D., Shamleffer, V. J., Jones, L. R., Studies pertaining to the antibacterial activity of sulfathiazole and its methyl derivative, *J. Bact., 44*:95–110, July 1942.
12. Poth, E. J., Ross, C. A., Studies on rates of hydrolysis of N^4 Dibasic acid substituted sulfonamides, *Proc. Soc. Exper. Biol. & Med., 57*:322–327, 1944.
13. den Dooren de Jong, *Bijdrage tot de Kennis van het Mineralisatie Disert*, Rotterdam, 1926. Quoted in Stephenson, Bact. Met., p. 189.
14. Braun, H., Cahn-Bronner, C. E., Der Verwendungstoffwechsel pathogener Bakterien, *Bioch. Zeitschr., 131*:272, 1922.
15. Quastel, J. H., On the fermentation of the unsaturated dicarboxilic acids, *Bioch. J., 18*:365, 1924.
16. McLeod, J. W., Gordon, M. B., The production of organic compounds of sulphur in bacterial cultures with special reference to glutathione, *Bioch. J., 18*:937, 1924.
17. Barron, E. S. G., *Adv. in Enzmology*, Vol. I-I, 149, New York 1943.
18. Trefouel, J., Nitti, F., Bovet, D., Chimiotherapie antibacterienne: rapport entre la constitution chimique et l'activite therapeutique des derives organiques du soufre, *Proc. Third Int. Congr. Microb.*, N. Y., p. 582, 1940.
19. Green, H. N., Bielschowsky, F., Mode of action of sulphanilamide III. Relation of chemical structure to bacteriostatic action of aromatic sulphur, selenium and tellurium compounds, *Brit. J. Exper. Path., 23*:13–26, Feb. 1942.
Rosenthal, S. M., Some non-sulphur compounds of interest in bacterial chemotherapy, *Proc. Third Int. Cong. Micr.*, p. 598–599, 1939.
Rosenthal, S. M., Bauer, H., Some relations of chemical structure to toxicity and to antibacterial action, *J. Bact., 39*:28, Jan. 1940.
20. Collier, H. B., Enzyme inhibition by derivatives of phenothiazine and of sulphanilamide, *Can. J. Res. B., 18*:345–350, Nov. 1940. Quoted in Henry's monograph.

Influence of Temperature and Medium Upon The Action of Sulfonamide

MICHELE GERUNDO, M.D., PH.D.

From the Department of Bacteriology, University of California, Los Angeles
and
The Department of Laboratories, Hilo Memorial Hospital, Hilo Hawaii

EXPERIMENTAL

It is not clear whether the action of sulfonamide is due to adsorption of the drug upon the bacterial cell, or to some different mechanism. These experiments were carried out with the view of throwing some light on this subject.

The first experiment was planned according to yielded data given on Table I. Three temperatures were selected at first: 37°, 28°C., and 4° or 5°. All tubes were inoculated at the same time with the same loop from the same uniform suspension. One set of tubes was incubated at 37°, another at 28°, and a third set kept for 12 hours at refrigerator temperature before being brought to 37°. It was considered that in the refrigerator no growth would take place, but that the drug would have time to adsorb on to the surface of the bacterial cells, thus making its bacteriostatic action more prompt and evident. It had been claimed[1] by certain authors that inhibition of growth is more effective in presence of a small inoculum or a smaller number of bacteria, although Nitti et al.[2] consider such factors variable according to the various micro-organisms. From Table I it is evident that the tubes left in the refrigerator for 12 hours before being incubated showed a definitely delayed growth as compared to when they were incubated at 37°C., even with such strains as *Eberthella typhi*, which is relatively resistant to sulfonamide. But whereas for example, in the case of *Eberthella typhi*, the 24 hours growth was similar in all three sets of tubes, *Proteus vulgaris* and *Shigella dysenteriae sonnei* showed a lighter growth in the refrigerated tubes than similar tubes incubated at 37°. On the other hand, *Aerogenes aerobacter* or *Salmonella typhi* showed no delay in growth. The incubation at 28° gave almost similar results, that is, a delayed growth in comparison with tubes incubated at 37°.

By culturing the bacteria on a solid medium, the results were practically identical to those obtained on fluid mediums.

Para-aminobenzoic acid prevented this delaying action of Sulfasuxidine in most instances. The exceptions were *Brucella abortus* and *Staphylococcus 12*. This last one grew in the presence of Sulfasuxidine and suxidine in most instances. The exceptions were Brucella abortus and Staphylococcus 12. This last one grew in the presence of Sulfasuxidine and para-aminobenzoic acid at 37°, but not in the tubes kept for 12 hours at refrigerator temperature before incubation. By using a synthetic medium,

TABLE I

TRYPTOSE PHOSPHATE GLUCOSE BROTH *p*H 7.3
SULFASUXIDINE 1:500
PABA 1:1000 5cc. FOR EACH 100cc. OF MEDIUM

	With Sulfasuxidine						*Without Sulfasuxidine*					
	No PABA			*PABA*			*No PABA*			*PABA*		
	37°	28°	R	37°	28°	R	37°	28°	R	37°	28°	R
Eberth. typhosa R.	xx xxx	? xxx	X xxx	xxx xxx	x xxx	xxx xxx	xxx xxx	x xxx	xxx xxx	xx xxx	x xxx	xx xx
Salmonella Schottmuelleri	XX xx	? xx	x xx	xxx xxx	xxx xxx	xxx xxx	xxx xxx	xx xx	xx xxx	xxx xxx	xxx xxx	xx xxx
Alkaligena 110	– x	? ?	– –	– xx	– x	– x	– xx	– x	– x	– xx	– x	– ?
Proteus vulgaris	x xxx	? xxx	? ?	xxx xxx	xx xxx	xxx xxx	xxx xxx	xx xxx	xx xx	xxx xxx	xx xxx	xx xx
Salm. typhi	xx	–	xx	xx	–	xx	xx	–	xx	xx	–	xx
Shigella Sonnei	xx xxx	– –	xx –	xxx –	– –	xx xx	xx –	– –	xx xxx	xx –	– –	xx –
Esch. coli comm. 10	xxx	–	xxx	xxx	–	xxx	xxx	–	xxx	xxx	–	xxx
Salmonella typhi	xx –	– –	xx –	xx xxx	– –	xx –	xx xxx	– –	xx –	xx xxx	– –	xx –
Aereog. Aerob.	xxx	–	xxx	xxx	–	xxx	xxx	–	xxx	xxx	–	xxx

Top reading after 12 hours growth. Bottom reading after 24 hours.

One set of tubes was incubated at 37°; another set was left in refrigerator for twelve hours after inoculation and then incubated at 37°; a third set was left for 12 hours at 28° and then transferred to incubator at 37°. Readings were made after twelve hours and twenty-four hours in incubator at 37°. In order to have comparative data, the tubes which had been set in incubator soon after inoculation were transferred after twenty-four hours to refrigerator in order to prevent any further growth.

the results were similar. Although the medium itself was not very suitable for the growth of many micro-organisms, like *Eberthella typhi*, it is clear that para-aminobenzoic acid antagonizes inhibition by sulfonamides. However, this antagonism is more evident at 37° than it is at lower temperatures which already exercise an unfavorable influence upon growth. In another experiment the conditions were reversed. As it is the current belief that the action of the drug is chiefly upon divided cells, after a previous incubation of 12 hours at 37° the tubes were transferred for another 12 hours to the refrigerator and then reincubated. With exception of *Aerogenes aerobacter* and *Proteus vulgaris* there was no significant difference between the two sets of tubes indicating that the action of the drug must take place before the bacteria divide and that, once the division has begun, the action of the drug is much decreased.

To demonstrate further the phenomenon of adsorption, the bacterial inoculum should be washed after preliminary contact with the drug, and resuspended in fresh medium. This experiment was not carried out, as some authors have pointed out[3] that sulfonamide can be washed out of the bacterial surface with the exception of small amounts which remain adherent to the bacterial body.

Discussion

Temperature doubtless plays a role in every biochemical process, so it is hardly a surprise that even in sulfonamide therapy it should hold a prominent place. A number of papers dealing with the influence of temperature upon the action of sulfonamide drugs in vitro show clearly that an increase in temperature enhances the bacteriostatic action of sulfonamides.[4] This phenomenon, of course, has some importance also in human therapy, as it indicates a more effective bacteriostasis during the febrile stages than in conditions not accompanied by fever.

In our investigations, however, the aim was not to test the effectiveness of the drug at various temperatures, but rather to understand its mechanism of action. Henry[5] stated that the reaction in which the sulfonamides are involved is an adsorptive one. Adsorptions are strongly exothermic and decrease in intensity with increasing temperature; however, no fast rule can be laid down since other factors are involved, which may definitely modify the influence of temperature. Kohn and Harris[6] found that low temperatures have no more effect upon bacteriostasis by sulfonamides after growth has occurred at a higher temperature.

Our experiments produced similar results. After growth had already begun during an incubation of 8 to 12 hours at 37°, sulfonamide inhibition was not increased by lowering the temperature. No attempt was made to culture at a higher temperature than 37°, as other unrelated variables would have been introduced. Our experiments seem to show clearly that lower temperatures influence bacteriostasis and that such influence may be of an adsorptive nature.

Whether the phenomenon may be one of diffusion into or adsorption

on the bacterial cell, is not clear as Feinstone, Williams and Florestano[7] have pointed out that inactive substances may enter or adhere without exercising any inhibition. However, these authors claim to have obtained evidence by means of the electron microscope that the drug does enter the cell. Although this evidence would explain the delay in action often encountered in experiments with sulfonamides in vitro, other authors[8] have found rather immediate influence of sulfonamide upon bacterial luminescence which, of course, speaks in favor of an adsorption condition.

Studies of influences of temperature upon delay of action of sulfonamide have been carried out by Kohn and Harris, Spring and Lowell and Finland.[9] These authors observed a lag in sulfonamide action at lower temperatures. From the experiments which were carried out in this laboratory it seems clear that sulfonamide inhibition was more pronounced by leaving the bacteria in contact with the sulfonamide at a temperature which did not initiate growth. Once growth had been initiated, inhibition of growth by refrigeration did not bring about any increased sulfonamide action. At 28° the inhibition also was more evident than at 37°. It does seem reasonable to conceive that the phenomenon is adsorptive in nature and that the adsorption must take place before metabolic activities of the bacteria and division of the cells have begun. In favor of adsorption also are the findings of Johnson and Moore[10] who reversed entirely sulfonamide inhibition by washing and resuspending the cells in a drug-free solution.

While it is possible that differences in results obtained by various authors may be explained by other factors involved, the experiments carried out are in favor of the adsorptive nature of the drug.

BIBLIOGRAPHY

1. Libby, R. L., Activity of chemotherapeutic agents, *J. Bact.*, *40*:733–745, Nov. 1940.

2. Nitti, F., Philippe, M., Bovet, D., Action rétardante de certains derivés organiques, du soufre, sur le developpement du bacille diphtérique. Phénom nes d'accoutumance. *Compt. rend. Soc. de biol*, *131*, 70–72, 1939.

3. Johnson, F. H., Moore, K., Sulfonamide inhibition of bacterial luminescence, *Proc. Soc. Exp. Biol. & Med.*, *48*:323–325, July 1941.

4. Hadley, P. B., F. P., Influence of sulfanilamide on mucoid and smooth-phase cultures of hemolytic streptococci *in vitro*, *J. Inf. Dis.*, *68*:246–263, May-June 1941.

White, H. J., Relationship between temperature and the streptococcicidal activity of sulfanilamide and sulfapyridine *in vitro*, *J. Bact.*, *38*:549–562, Nov. 1939.

Neter, E., Effect of temperature on bacteriostatic action of sulfanilamide upon members of enterococcus group, *Proc. Soc. Exper. Biol. & Med.*, *43*:52–55, Jan. 1940.

Lee, S. W., Epstein, J. A., Foley, E. J., Temperature and bacteriostatic action of sulfathiazole and other drugs II. Streptococcus pyogenes, *Proc. Soc. Exper. Biol. & Med.*, *53*:245–247, June 1943.

Bliss, E. A., Long, P. H., Observations on the mode of action of sulfanilamide, *J. Am. Med. Assn.*, *109*:1524–1528, Nov. 16, 1937.

5. Henry, R. J., The mode of action of sulfonamides, p. 170, *J. Macy Jr. Foundation*, 1944.

6. Kohn, H. I., Harris, J. S., On mode of action of sulfonamides I. Action on Escherichia coli, *J. Pharm. & Exper. Therap.*, *73*:343–361, Nov. 1941.

7. Feinstone, W. H., Williams, R. D., Florestano, H. J., Studies on the action of sulfonamides upon the bacterial cell, *J. Bact.* *53*:75, Jan. 1942.

8. Johnson, F. H., Chase, A. M., Sulfonamide and urethane inhibition Cypridine luminescence *in vitro*, *J. Cell. Comp. Phys.*, 19:151–152, 1942.

Johnson, F. H., Eyring, H. B., Nature of the changes in optimal temperature of luminescence

caused by enzyme-sulfanilamide and enzyme-urethane equilibria, *J. Bact.*, *45*:24, Jan. 1943.

Johnson, F. H., Moore, K., Sulfonamide inhibition of bacterial luminescence, *Proc. Soc. Exper. Biol. & Med.*, *48*:323–325, 1941.

9. Kohn, H. I., Harris, J. S., On mode of action of sulfonamides I. Action on Escherichia Coli, *J. Pharm. & Exper. Therap.*, *73*:343–361, Nov. 1941.

Quoted: Spring, W. C., Jr., Lowell, F. C., Finland, M., Studies on action of sulfapyridine on pneumococci, *J. Clin. Inv.*, *19*:163–177, Jan. 1940.

10. Johnson, F. H., Moore, K., Sulfonamide inhibition of bacterial luminescence, *Proc. Soc. Exper. Biol. & Med.*, *48*:323–325, 1941.

Sustained Blood Streptomycin Levels Following Depot Administration of The Ferriheme and Oleocarbonate Salts

ROBERT D. BARNARD, M.D.

with the technical collaboration of
HERBERT A. WEITZNER, M.D., GEORGE B. GORDON, M.D. AND ISADORE ROSSMAN, M.D.
New York, N. Y.

While some discussion now attends the merit formerly attached to sustained blood levels of systemically administered antibiotics, it is nevertheless a fact that repository or depot forms of streptomycin, unlike their penicillin counterpart, have not been successfully evolved. Kolmer and his associates reported on the administration of suspensions of streptomycin in oily menstruums but detectable blood levels beyond a few hours after intramuscular deposition, were not secured. The levels reported by Kolmer et al[1] do not differ significantly from those observed after a single intramuscular injection of the aqueous streptomycin solutions.

Depot administration forms of insulin[2] and various alkaloids[3], based on coordination compounds of the former, with ferriheme (hematin) have been described. It has recently been determined that guanidyl derivatives form polar covalent compounds with the ferriheme radical[4]. On the basis of the presence of the guanidyl-imino groups in the streptomycin molecule, an attempt was made to utilize such a coordination compound in the preparation of a repository form of this antibiotic. By reacting streptomycin sulfate in aqueous solution with two molar equivalents of lithium ferriheme (the latter being water soluble at neutrality[5]) an insoluble reaction product containing the streptomycin moiety, was prepared. The brownish black precipitate, when resuspended in water and injected intramuscularly into rabbits and dogs, resulted in a series of protracted blood levels. The values in rabbits are rendered in Table I. In dogs, detectable streptomycin levels in the blood were found in some instances four days after a single intramuscular injection of 250,000 units (as streptomycin base) of the ferriheme derivative.

Though in no instances did there appear to be local induration or evidence of undue pain in the injected animals, and although suspensions of streptomycin ferriheme passed satisfactory safety and sterility tests, its use in human patients was precluded by the high volume-dosage ratio of the aqueous conjugate suspension. It was found impossible to prepare a readily syringible material of more than 100,000 units (as streptomycin base) per cc. of suspension.

While attempting circumvention of the last difficulty by milling the dried streptomycin-ferriheme conjugate in oil, it was noticed that this conjugate dispersed more like a wax than like either the ferrihemochromogen or ferrideheme that the guanidyl coordination would mandate.[6] It was obvious that the ferriheme had entered into combination with the streptomycin, for the major part at least, as a dibasic aliphatic acid and that the conjugate was actually an ester.

On this basis, various streptomycin waxes, including the oleate[7], palmitate and oleocarbonate were prepared. The oleocarbonate of dihydrostreptomycin was found particularly suitable for experimental depot intramuscular administration to animals. It is a faintly colored solid which disperses readily in vegetable oils and the dispersate is readily syringible through a 20 gauge needle in doses of 250,000 units (as streptomycin base) per cc. Table II gives representative blood levels secured following the intramuscular injection into rabbits of dihydrostreptomycin oleocarbonate in sesame oil.

While these levels appeared to be satisfactory insofar as any criteria that might be imposed by those wishing repository streptomycin effects, the material was not administered to humans. Again, the manner of dispersion in oil gave a clue as to covert properties for the oleocarbonate at least one of which was felt to be undesirable.*

The oleocarbonate, on standing in contact with oil, would lose opacity and this change was accompanied by a loss of CO_2 and reversion to a liquid suboleate. Since the properties of neither the oleocarbonate nor the suboleate of streptomycin or dihydrostreptomycin had appeared in the literature, they were subjected to further study. The oleocarbonates would revert to the suboleates on standing; the reversion was quite rapid at 50 – 60° C. without loss of antibiotic potency. The suboleate was found to have a marked "tweening" effect similar to the recently introduced (clinically) sorbitan monoleate polyoxyethylene derivative now established as a therapeutic modality for secural of extra-portal intestinal absorption of lipoid substances. That this property should likewise be inherent in the streptomycin suboleates immediately suggested their use as *oral* repository dosage forms.

Administration to humans has proved their efficacy in this connection and the therapeutic application of this principle to antibiotic and other types of therapy will be the subject of a separate communication.

Summary

Repository effects of the ferriheme and oleocarbonates of streptomycin are described along with some of their physical and chemical properties.

*Even if such properties had not become manifest, it is doubtful whether, under any circumstance, the oily streptomycin repository would have been injected into humans. The author, for some time, has been opposed to extension of oil repository materials into therapeutics, feeling that in the case of the penicillin repositories at least, they have been responsible for some degree of the broad "spectrum" of hypersensitivities that is now exhibited by the American public.

TABLE I

BLOOD LEVELS OF STREPTOMYCIN**

Animal	*Dosage**	*Hours after Administration of Streptomycin Ferriheme*									
		1	*½*	*8*	*12*	*16*	*24*	*36*	*48*	*60*	*72*
1	12	1 7	—	1 1	—	0 9	—	0 3	—	n	—
2	15	3 1	—	—	—	0 4	—	n	—	—	—
3	33	8 1	—	8 8	—	—	3 2	—	1 2	—	d
4	48	6 4	7 1	—	4 2	—	—	—	2 9	1 0	d
5	110	14 0	—	—	—	—	3 3	—	1 5	—	0 6
Control***	100	25 0	2 8	d	n	—	—	—	—	—	—
Control	100	16 6	1 3	n	—	—	—	—	—	—	—
Control	200	37 0	10 2	d	n	—	—	—	—	—	—

*In milligrams per kilo of streptomycin base
**In micrograms of streptomycin base per cc. of blood
d—Detectable but not amenable to estimation
n—Examined for but undetectable
***Aqueous solution of streptomycin sulfate, 1 cc. = 200 milligrams of streptomycin base.

TABLE II

BLOOD LEVELS OF DIHYDROSTREPTOMYCIN

Animal	*Dosage*	*Hours after Administration of Dihydrostreptomycin Oleocarbonate*					
		1	*12*	*24*	*48*	*72*	*96*
11	100	5 3	8 2	8 7	3 3	d	n
12	72	13 3	5 0	2 7	d	d	n
13	300	17 0	31 0	11 0	5 5	0 8	d
14	300	21 0	18 5	15 0	7 0	0 7	d
Control***	300	150 0	d	d	n	–	–

Designations as in Table I
***Aqueous solution of Dihydrostreptomycin hydrochloride 1cc. = 166 milligrams of streptomycin base

REFERENCES

1. Kolmer, J. A., Bondi, A., Warner, H. F., and Dietz, C. C., Depot Form of Streptomycin, *Science*, *104*, 317, 1946.
2. Barnard, R. D., Insulin Ferriheme chromogen, *J. Lab. & Clin. Med.*, *27*, 774, 1942.
3. Barnard, R. D., Therapeutically Active Ferriheme chromogen, U. S. Patent 2, 364, 018, Nov. 28, 1944.
4. Barnard, R. D., Guanidine Ferriheme and the Carbimide Structure of "Cyanamide," *Ohio State J. Science*, 48, 189, 1948.
5. Barnard, R. D., Saccharine and Lithium Ferriheme on Blood Coagulation, *J. Am. Pharm. Assn.* 36, 183, 1947.
6. Barnard, R. D., Color Reaction of Cyanamide and Ferriheme; Mechanism of Action of Certain Tissue Poisons, *J. Am. Pharm. Assn.*, *33*, 24, 1944.
7. Jadassohn, W., Cherdiuliz, E., Boymond, P., and Isler, H., Insoluble Antibiotics, *Experientia*, *4*, 225, 1948.

News, Notes and Editorial Comments

New Potent Drug for Amebiasis

High amebacidal potency of Milibis, a newly developed drug, as compared with other available compounds for the treatment of amebiasis has been demonstrated both clinically and in laboratory experiments, according to a report published in the *American Journal of Tropical Medicine* by Doctors E. W. Dennis and D. A. Berberian and Sophie S. Hansen.

The clinical experiments were conducted by Dr. Berberian at the American University, Beirut, Lebanon. All of 72 patients with subacute amebiasis were promptly cleared of ameba by oral doses of 0.5 Gm. Milibis taken in daily doses for seven to ten days without any signs of toxicity. This series included 31 patients who were given Milibis as the only amebacide. There were only three recurrences among the 31 patients during a follow-up period of 287 days.

Laboratory experiments by the authors at the Sterling-Winthrop Research Institute showed that Milibis was superior to other amebacidal drugs when tested against *E. histolytica* in Hansen's egg-infusion medium.

Milibis in a daily dose of 75 mg. kg for five days cleared 10 hamsters of amebiasis infection. Ten times the effective dose of Milibis given for five days caused no signs of toxicity in these animals, an observation in striking contrast to the toxicity of effective doses of other amebacides.

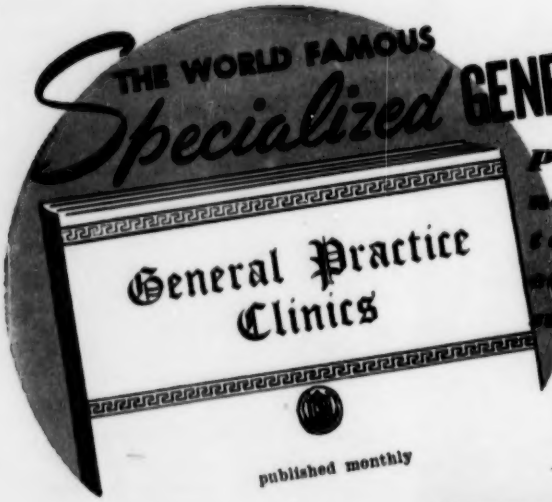
New Package for Neocurtasal

Neocurtasal, the seasoning agent for salt sodium-free diets produced by Winthrop-Stearns Inc., is now available in unit service packets in strips of five, in addition to the 2-ounce shaker container and the 8-ounce bottles already available.

The new package is of special interest to hospitals for serving patients on trays. It is also convenient for travelers, who wish to carry the small unit packets with them.

THE WORLD FAMOUS

Specialized GENERAL JOURNAL



published monthly

Presenting the newer and better technics from every medical and surgical center.

SCIENTIFICALLY ACCURATE.
CLINICALLY AUTHORITATIVE.
ACCEPTED THE WORLD OVER.

Each issue of General Practice Clinics presents a concise and authoritative description of the important tried, proved and accepted; new and better clinical methods in:

MEDICINE
ALLERGY
GERIATRICS
PSYCHIATRY
NEUROLOGY
DERMATOLOGY AND SYPHILOLOGY
PEDIATRICS
OBSTETRICS
GYNECOLOGY
SURGERY
ORTHOPEDICS
UROLOGY
OPHTHALMOLOGY
OTORHINOLARYNGOLOGY
MEDICAL JURISPRUDENCE

Every General Physician and Specialist will find on these pages the very latest and best clinical methods now being successfully used at every medical and surgical center. Specific detailed dosages, exact information which you may safely and successfully employ in your own practice—all of these data are compiled under the personal direction of eminent authorities—whose abilities and reputations are well known.

WASHINGTON INSTITUTE OF MEDICINE, 1720 M Street, N. W., Washington 6, D. C.